Correlations of *Vibrio fischeri* Bacteria Test Data with Bioassay Data for Other Organisms

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Linear relationships of the median lethal concentrations of several hundreds of chemicals for a variety of organisms with *Vibrio fischeri* median effective concentrations are investigated. Significant correlations can be developed for many aquatic species including the fishes fathead minnow, bluegill, catfish, goldfish, goldorfe, guppy, killifish, rainbow trout, sheepshead minnow, and zebrafish; the water flea *Daphnia* sp.; such crustaceans as *Artemia* sp. and *Crangon* sp.; the ciliate *Tetrahymena pyriformis*; and algae, such as *Chlorella* sp. These interspecies relationships can be used to estimate order-of-magnitude type toxic effects of many substances for these aquatic organisms. Highly significant relationships can be obtained when selecting compounds on a chemical basis, such as alcohols, ketones, aromatics, etc., which allow the calculation of the compounds' toxicities to the corresponding aquatic species with increased accuracy and confidence. Analogous correlations with mammalian (rat and mouse) oral, intraperitoneal, and intravenous median lethal dose (LD_{50}) data are much weaker than those for most aquatic species. However, there are significant differences between these three routes of administration and the intravenous LD_{50} data show the best relationship with the *Vibrio* data. — *Environ Health Perspect* 106(Suppl 2):583–591 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/583-591 kaiser/abstract.html*

Key words: *Vibrio*, bioassay, fish, *Daphnia*, crustacea, acute toxicity, rat, intravenous, dose

Introduction

The rising interest in finding alternatives to using large scale, expensive, and time-consuming aquatic and terrestrial species tests for chemicals and commercial products has led to the investigation of several alternatives. One promising alternative is the use of bacteria for this purpose, especially *Vibrio fischeri*, formerly known as *Photobacterium phosphoreum* and commonly referred to as the Microtox test (Azur Environmental, Carlsbad, CA).

The photoluminescent bioassay uses a suspension of *V. fischeri* bacteria in saline water (to protect this marine bacterium from osmotic damage) and measures the reduction in light output of its natural luminescence on exposure to the toxicant of interest. In contrast to most aquatic bioassays, in which acute toxicities are usually measured over a period of 96 hr, the bacteria test can be performed in a matter of minutes. Furthermore, dormant bacteria concentrates can be kept frozen and ready for use for a prolonged period. Therefore, this test has generated widespread interest and has been adopted as a standardized test in several jurisdictions. It can be performed on demand in the laboratory without the need for keeping fish or mammals in larger scale facilities for extended periods. This convenience is obviously accompanied by savings in cost and time. Materials and specialized spectrophotometers are readily available from several commercial suppliers of the luminescence bioassay test systems, which are marketed under the names Microtox (Azur Environmental), Lumistox (Dr. Lange GmbH, Berlin, Germany), and Biotox (BioOrbit, Turku, Finland).

Considerable toxicity data for *V. fischeri/P. phosphoreum* are available in the form of monographs (*1,2*) covering over 1200 chemicals. More recently, interactive databases have become available on CD-ROM. These CD-ROMs are marketed under the names Computox (Environment Canada, Ottawa, Ontario), which covers 1500 substances (*3*), and TerraTox (TerraBase Inc., Burlington, Ontario), which covers over 1700 substances (*4*), as well as specialized software for data analysis and toxicity estimation (*4*) available under the name TerraFit (TerraBase Inc.) Furthermore, symposium proceedings dealing with the use of photoluminescent bacteria for ecotoxicologic monitoring have been published (*5*). It is not surprising then that the *V. fischeri/P. phosphoreum* data have become the largest published toxicity test data set for a single aquatic species.

The biochemical mechanisms by which compounds exert a toxic or bioluminescence-reducing effect on the bacterium are only partly understood. In principle, the enzyme luciferase catalyzes the oxidation of reduced riboflavin phosphate, which is accompanied by emission of light. This process is linked with the microbial metabolism, and hence is directly linked to the toxic effect of a substance on the bacterium (*6*). Although different toxic mechanisms may exist in other organisms, substances highly toxic in one organism often also produce effects on quite different organisms. Isenberg, one of the original developers of the photoluminescent bacterial bioassay, describes this as testimony to the unity of life and shows that the median effective concentration (EC_{50}) values for a variety of anesthetic gases are very similar for bacteria, mouse, dog, cat, and human (*6*).

Several studies have dealt with the relative sensitivity and comparability of the luminescent bacteria test with other, mostly aquatic, bioassays. Because this bioassay can be performed with relative ease and speed and at a limited cost, it is of particular interest as a test vehicle for routine monitoring once good correlations with local species have been established. A growing number of publications also use it for the ecotoxicologic assessment of contaminated soils and sediments and as a monitoring tool for site remediation. In research and product development studies, this test allows the rapid screening of novel compounds or a series of related compounds for their relative biologic activity.

Given the large number of available data and the simplicity and robustness of the test, it is of much interest to undertake

qualitative and quantitative comparisons of the Microtox test data with those of other bioassays, particularly aquatic species. A variety of earlier studies on this subject include the works by Kaiser et al. (7), Zhao (8), Kaiser (9), Fort (10), Munkittrick et al. (11), Bulich et al. (12), Xu et al. (13), Dutka and Kwan (14), Maas-Diepeveen and van Leeuwen (15), Nacci et al. (16), Tarkpea et al. (17), Greene et al. (18), DeZwart and Slooff (19), Ribo and Kaiser (20), Qureshi (21), and Curtis (22). Given the multitude of species, bioassays, and chemical substances, this study emphasizes those species and bioassays where larger numbers of data are in common, perusing the largest available data compilation (4).

A Nonexhaustive Review of Available Information

A number of studies have undertaken comparisons of the *P. phosphoreum* test with other bioassays, including other test species, length of exposure, type of effect, etc. A detailed list of such comparisons is given in Kaiser and Palabrica (1); an extract of this review is updated in Table 1.

Although not all of the above investigations allow quantitative comparisons, numerous data allow interspecies comparisons between *V. fischeri* and acute, subchronic, or chronic tests for a number of other aquatic species. In addition, recent research by Azur Environmental has resulted in the development of a chronic *V. fischeri* bioassay as well as a mutagenicity assay using a dark mutant of the organism. The *V. fischeri* toxicity assay, commonly known as the Microtox assay, is becoming a standardized toxicity assay in several countries (5), including Canada (23), France, Germany, Italy, Mexico, the Netherlands, Spain, Sweden, and the United States (24).

Data Sources, Notation, and Treatment

The data used here are those found in the handbook on *Ecotoxicity of Chemicals to Photobacterium phosphoreum* (2), augmented with new measurements (25), and other information and data as found in the Computox database (3) and the Terra-Tox/TerraFit software suite (4). All toxicity data used here are given in the pT notation, where $pT = \log [(MW/(mmol/liter)]$ for concentration-derived values, and $pT = \log [MW/(mg/kg$ body weight)] for lethal dose values, where MW is molecular weight. Details on this conversion are discussed in Kaiser and Palabrica (1). Preference was

Table 1. Aquatic and terrestrial organisms for which qualitative and/or quantitative bioassay correlations with *P. phosphoreum* or *V. fischeri* have been investigated.

Organism	Species	Recent reference
Bacteria	Spirillum volutants	Qureshi et al. (21)
	Nitrosomonas and Nitrobacter sp.	Maas-Diepeveen and van Leeuwen (15)
	Escherichia coli	Xu et al. (13), Rychert and Mortimer (50)
	Staphylococcus aureus	Devillers et al. (38)
	Streptococcus faecalis	Devillers et al. (38)
	Pseudomonas fluorescens	Xu et al. (13)
	Aeromonas hydrophila	Dutka and Kwan (14)
	Pseudomonas putida	DeZwart and Slooff (19)
	Microcystis aeruginosa	DeZwart and Slooff (19)
	Bacillus subtilis	Ribo and Kaiser (20)
Yeasts	Pichia sp., Rhodotorula sp.	Ribo and Kaiser (55)
	Saccharomyces cerevisiae	Xu et al. (13), Ribo and Kaiser (55)
Protozoa	Entosyphon sulcatum, Uronema parduczi	DeZwart and Slooff (19)
	Chilomonas paramecium	DeZwart and Slooff (19)
	Colpidium campylum	Vasseur et al. (39)
	Tetrahymena pyriformis	Kaiser and Esterby (40)
Algae	Selenastrum capricornutum	Blaise and Harwood (41), Miller et al. (42)
	Scenedesmus quadricauda	Kaiser and Esterby (40)
	Scenedesmus pannonicus	DeZwart and Slooff (19)
Coelenterate	Hydra oligactis	DeZwart and Slooff (19)
Rotifer	Brachionus plicatilis	Snell et al. (45)
Oyster	Crassostrea gigas	Becker et al. (46)
Snail	Lymnea stagnalis	DeZwart and Slooff (19)
Crustaceans	Artemia salina	Devillers et al. (38)
	Crangon septemspinosa	Ribo and Kaiser (20)
Cladocerans	Daphnia magna	Kaiser and Esterby (40), Miller et al. (42), Deneer et al. (43), Bazin et al. (44), Gälli et al. (47)
	Daphnia pulex	DeZwart and Slooff (19)
	Cerodaphnia dubia	Hoke et al. (36), Ankley et al. (48)
Copepod	Nitocra spinipes	Tarkpea et al. (17)
Amphipod	Rhepoxynius abronius	Becker et al. (46)
Insects	Chironomus tentans	Hoke et al. (36)
	Aedes aegypti	DeZwart and Slooff (19)
	Culex pipiens	DeZwart and Slooff (19)
	Hexagenia limbata	Hoke et al. (36)
Sea urchin	Arbacia punctulata	Nacci et al. (16), Snell et al. (45)
Fish		
Guppy	Poecilia reticulata	van Leeuwen et al. (49)
Carp	Cyprino carpio	Zhao et al. (8)
Goldorfe	Leuciscus idus melanotus	DeZwart and Slooff (19), Blaise and Harwood (41), Birkholz et al. (51), Indorato et al. (52)
Rainbow trout	Oncorhynchus mykiss	DeZwart and Slooff (19), Blaise and Harwood (41), Birkholz et al. (51), Indorato et al. (52)
Brown trout	Salmo trutta	Indorato et al. (52), Ribo and Kaiser (20)
Bluegill	Lepomis macrochirus	Ribo and Kaiser (20), Indorato et al. (52)
Sheepshead minnow	Cyprinodon variegatus	Ribo and Kaiser (20)
Zebrafish	Brachydanio rerio	Devillers et al. (38)
American flagfish	Jordanella floridae	Holdway et al. (53)
Japanese medaka	Oryzias latipes	DeZwart and Slooff (19)
Fathead minnow	Pimephales promelas	Kaiser and Palabrica (1), Kaiser (9), Curtis et al. (22), Kaiser et al. (33), Indorato et al. (52)
Terrestrial species		
Frogs	Xenopus laevis	DeZwart and Slooff (19)
	Ambystoma mexicanum	DeZwart and Slooff (19)
Earthworm	Eisenia foetida	Miller et al. (42)
Plants		
Wheat	Triticum aestivum	Miller et al. (42)
Lettuce	Lactuca sativa	Miller et al. (42)
Radish	Raphanus sativa	Miller et al. (42)
Clover	Trifolium pratense	Miller et al. (42)
Cucumber	Cucumis sativa	Miller et al. (42)
Mammals		
Mouse		Kaiser et al. (7), Fort (10), Benson and Stackhouse (54)
Rat		Kaiser et al. (7), Fort (10), Kaiser and Esterby (40)

given to 30-min EC_{50} data over 15-min data, and 15-min EC_{50} over 5-min data, where available. All available data were used for the development of the correlations presented here, i.e., no preselection of any kind was made.

For the data analysis and graphing, the TerraTox/TerraFit software suite (4) was used. This program allows automated trimming of data pairs exceeding defined specifications. In this work, data removed in the trimming process are those for which the measured value differed by more than 2σ from the regression-derived estimated value for the end point complementary to the bacteria value. This corresponds roughly to a 95% confidence interval. The number of compounds so removed are given in the figure captions.

The data quality for all of the different end points in this paper varies widely and is uncertain in several instances. This variation stems both from the specifics of each test system used, for example, static systems with nominal concentrations and no consideration of abiotic degradation or loss through volatilization, and lack of detailed information on certain experimental conditions, such as pH, purity of chemicals tested, and related aspects. Therefore, in many instances confidence in the data themselves varies; for some of the data, the accuracy may vary by 0.5 orders of magnitude or more from values that would be found with more elaborate test systems and conditions for the same end point. Some comments on data quality and erroneous data are provided by Kaiser (26).

Correlations with Fathead Minnow Acute Toxicity Data

One of the largest single species aquatic test data sets, the 96-hr acute lethal concentration bioassay data for fathead minnow (*Pimephales promelas*), has data for approximately 800 chemicals, most of which have been measured by the Center for Lake Superior Environmental Studies, University of Wisconsin-Superior (Superior, WI) (27–31). At present there are published Microtox data for approximately 1700 compounds; the majority of these values originated at the National Water Research Institute, Burlington, Ontario. Between these two data sets there are now in excess of 400 individual compounds in common and their respective toxicities span 10 orders of magnitude on a molar basis.

Figure 1 gives a plot of these fathead minnow and Microtox toxicity data after

trimming compounds of $> 2\sigma$, which reduces the number of data pairs from 439 to 420. Table 2 gives the statistical details for this and other correlations and Table 3 shows a list of the compounds removed by trimming with their respective values. They include silver nitrate (which is difficult to determine in the Microtox test because of the normal presence of chloride ion for osmotic protection for the bacteria); four chemicals with terminal $= CRR'$ groups, where R or $R' = H$, Cl, which are recognized gill membrane irritants (vinylcarbazole, resmethrin, telone II, and allyl alcohol); three amines for which the pH has a critical influence on the apparent toxicity (ethylaniline, ethanolamine, and triethanolamine); several highly specific pesticides (DDT, dieldrin, endosulfan, aldicarb, fenvalerate, terbufos); and four other compounds for which no explanation appears readily available (two esters, one acid, and one phenol derivative). The observed fish toxicity for the amines and oleic acid was significantly lower than predicted from the equation in Table 2; for the other compounds in Table 3, observed fish toxicity was significantly higher.

Figure 1 shows a strong correlation between the fathead minnow median lethal concentration (LC_{50}) and bacteria EC_{50} values over a toxicity range of over 10 orders of magnitude in molar activity. The entire set is collated strictly on the basis of compounds measured in both tests without any other selection process. Although there

is a considerable standard deviation of 0.83 pT units, this correlation is sufficiently strong to use it for a quick order-of-magnitude estimation of fish toxicities from those found in the Microtox test. This correlation can be applied to many substances because it also covers a large variety of chemical classes, including alcohols and phenols, aliphatic, aromatic, and heterocyclic compounds, acids, ketones, nitriles, nitro-, fluoro-, chloro-, and bromo-substituted chemicals, and various pesticides, including fungicides, herbicides, insecticides, and others. Where more reliable predictions are desired, selection of the chemicals by class, mode of action (if known), or similar functions will be required to increase the quality of the regression and reduce the standard deviation of the estimate. Such an approach can produce significant improvements and has been successfully demonstrated for many series of homologs for these and other species; some examples are given below.

Using this information and the now well established relationship of Microtox and fathead minnow data, we can make predictions for the latter for compounds that have not been directly measured in that system. For example, the TerraFit prediction system (4) allows the easy computation of approximately 1200 fathead minnow toxicity (pT) data. Of these, values in excess of 4.00 (i.e., highly toxic substances) can be calculated for busan, chloroxuron, several tributyltin derivatives,
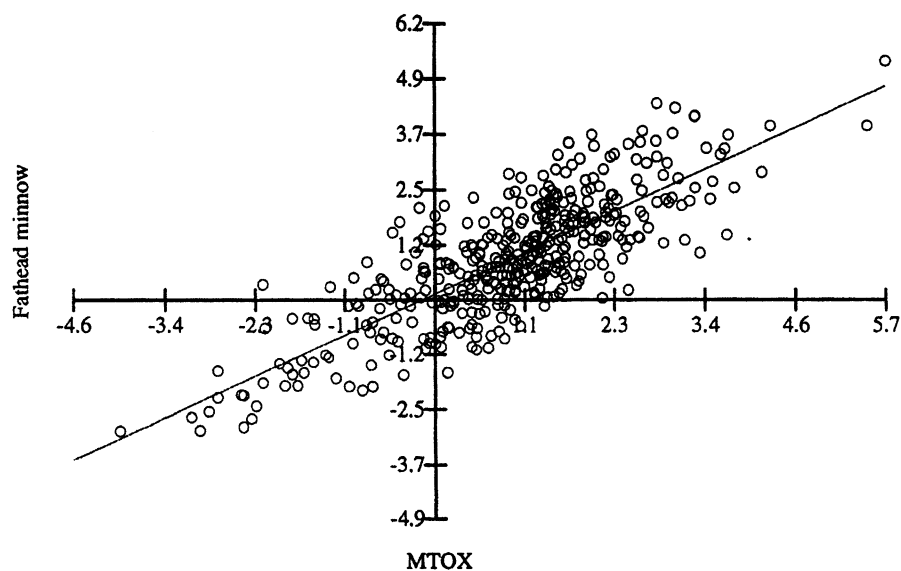

Figure 1. Plot of the acute toxicities of 420 individual organic chemicals in the fathead minnow 96-hr LC_{50} test (FHM) versus the luminescent bacteria 5-min to 30-min EC_{50} test (MTOX). Statistics are in Table 2; number of data trimmed, 19.

Table 2. Linear correlations of Microtox toxicity data with various fish toxicity data for the equation Microtox = $a + b$ (species). All data in pT units.

Species/time, hr	n	r	s	a	b	Range	Reference	Comments
Fathead minnow/96	201	0.81	0.81	−0.00	0.78	10	Kaiser and Palabrica (1)	
Fathead minnow/96	216	0.85	0.72	−0.08	0.81	10	Kaiser (9)	Trimmed
Fathead minnow/96	439	0.74	1.00	0.22	0.80	10	This paper	Untrimmed
Fathead minnow/96	420	0.80	0.83	0.14	0.81	10	This paper	Trimmed (<2σ)
Fathead minnow/96	398	0.84	0.73	0.08	0.84	10	This paper	Trimmed twice
Bluegill/96	184	0.71	1.24	0.70	0.88	10	This paper	Untrimmed
Bluegill/96	175	0.76	1.04	0.56	0.87	10	This paper	Trimmed (<2σ)
Catfish/96	83	0.76	0.93	0.63	0.74	8	This paper	Trimmed (<2σ)
Goldfish/96	53	0.85	0.76	0.10	1.00	6	This paper	Trimmed (<2σ)
Goldorfe/96	102	0.91	0.62	−0.10	0.88	9	This paper	Trimmed (<2σ)
Guppy/96	45	0.87	0.68	0.03	0.93	6	Kaiser (9)	Trimmed
Guppy/96	97	0.75	0.94	0.41	0.75	7	This paper	Trimmed (<2σ)
Rainbow trout/96	43	0.76	0.96	0.24	0.84	10	Kaiser (9)	Trimmed
Rainbow trout/96	174	0.83	0.89	0.56	0.89	10	This paper	Trimmed (<2σ)
Red killifish	44	0.77	0.65	0.33	0.73	6	This paper	Trimmed (<2σ)
Sheepshead minnow/96	53	0.74	0.80	0.51	0.80	6	This paper	Trimmed (<2σ)
Zebrafish/96	54	0.72	0.62	0.31	0.65	5	This paper	Trimmed (<2σ)
FISH-mix	647	0.75	0.97	0.26	0.81	10	This paper	Untrimmed
FISH-mix	635	0.81	0.80	0.17	0.81	10	This paper	Trimmed (<2σ)

Abbreviations: a, intercept of correlation; b, slope of correlation; FISH-mix, fish composite EC_{50} values; n, number of data pairs; r, correlation coefficient; range, orders of magnitude covered by the species values; s, standard deviation.

Table 3. List of chemicals and their MTOX and FHM toxicity values removed by trimming (<2σ) of total data set.

Compound	MTOX	FHM
4-Ethylaniline	2.76	0.22
Oleic acid	2.69	0.14
Silver nitrate	2.39	4.28
Terbufos	1.96	4.34
N-vinylcarbazole	1.90	4.78
Fenvalerate	1.87	6.00
p,p'-DDT	1.76	4.46
Endosulfan	1.53	5.43
Resmethrin	1.39	4.74
2,4,6-tri-tert-Butylphenol	1.33	3.63
Dieldrin	1.27	4.33
Diphenyl phthalate	0.71	3.60
Ethanolamine	0.65	−1.53
Aldicarb	0.40	3.83
Telone II	0.19	2.67
Triethanolamine	−0.12	−1.90
Allyl alcohol	−0.99	1.90
Dimethyl malonate	−1.83	1.03

Abbreviations: FHM, fathead minnow; MTOX, Microtox.

tetraoctyltin, benzyl isothiocyanate, and other compounds. Similarly, some compounds for which very low toxicity to fathead minnow (pT < −2.00) is predicted are propanediol and -triol, alanine, ethylamine, urea, and methyl carbamate.

Correlations with Acute Toxicity Data for Other Fish Species

Table 2 also gives the regression statistics for a variety of linear correlations between Microtox and other fish acute toxicity bioassay data. The results are given for bluegill (*Lepomis macrochirus*), 96-hr LC_{50}; channel catfish (*Ictalurus punctatus*), 96-hr LC_{50}; goldfish (*Carassius auratus*), 96-hr LC_{50}; goldorfe (*Leuciscus idus melanotus*), 48-hr LC_{50}; guppy (*Poecilia reticulata*), 96-hr LC_{50}; rainbow trout (*Oncorhynchus mykiss*), 96-hr LC_{50} (Figure 2); red killifish (*Oryzias latipes*), 24- to 96-hr LC_{50}; sheepshead minnow (*Cyprinodon variegatus*), 96-hr LC_{50}; and zebrafish (*Brachydanio rerio*), 96-hr LC_{50}, together with some correlations on smaller data sets from the literature. The new correlations are for data sets ranging from 44 (red killifish) to 439 (fathead minnow) data pairs and cover a toxicity range of 5 (zebrafish) to 10 (several species) orders of magnitude on a molar basis.

Table 2 also gives the correlation of *V. fischeri* EC_{50} values with fish composite values. This composite is made up of the arithmetic mean pT value of all individual fish LC_{50} data (pT values) where available in the database, using a limit of 1 pT value per end point (*32*) and all available Microtox pT values. This correlation covers 647 individual chemicals and 10 orders of magnitude each for the fish and Microtox values. The highly significant correlation (*p* < 0.001) obtained after one trimming, covering 616 compounds, is shown in Figure 3. The slope for the composite fish regression (*b*= 0.81, Table 2) is identical to that for the fathead minnow regression (trimmed once) and approximately in the middle of the range of the slopes of all individual fish species regressions, an indication of the generality of these relationships. Furthermore, as the different fish species analyzed include cold water (e.g., rainbow trout), warm water (e.g., fathead minnow), and tropical fish (zebrafish), as well as marine- (sheepshead minnow) and freshwater-inhabiting species (all others), the results indicate a wide range of applicability of these *Vibrio* to fish correlations for the order-of-magnitude estimation of the acute toxicity of chemicals. As mentioned for the fathead minnow, for more accurate estimations and reduced standard deviations, selection of smaller sets of relevant chemicals by mode of action, chemical class, or other proximity indicators should precede the analysis and prediction. In this field, attempts are presently underway to develop and apply more powerful algorithms, including multiple linear correlations and neural networks to improve the predictive capabilities of these correlations (*33,34*).

Correlations with Toxicity Data for Other Aquatic Organisms

A detailed list of correlations of *V. fischeri* EC_{50} values with acute toxic values to numerous organisms is given in Table 1. Besides the relationships for several fish species, explored in some detail above, a considerable volume of data allows the analysis of interspecies correlations between this bacterium and other aquatic organisms. Table 4 gives several regressions developed for an alga (*Chlorella pyrenoidosa*), 96-hr EC_{50}; the water flea (*Daphnia magna*), 48-hr LC_{50}; the crustacean (*Artemia salina*), 24-hr LC_{50}; the crustacean (*Crangon* sp.), 96-hr LC_{50}; the crayfish (*Oronectes immunis*), 96-hr LC_{50};
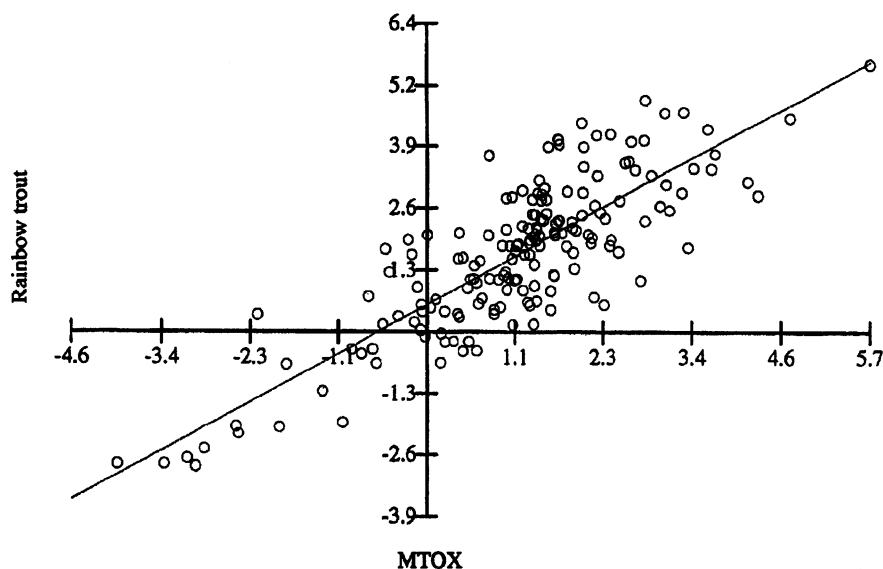
Figure 2. Plot of the acute toxicities of 174 individual organic chemicals for the rainbow trout 96-hr LC_{50} test versus the luminescent bacteria 5-min to 30-min EC_{50} test (MTOX). Statistics are in Table 2; number of data trimmed, 9.
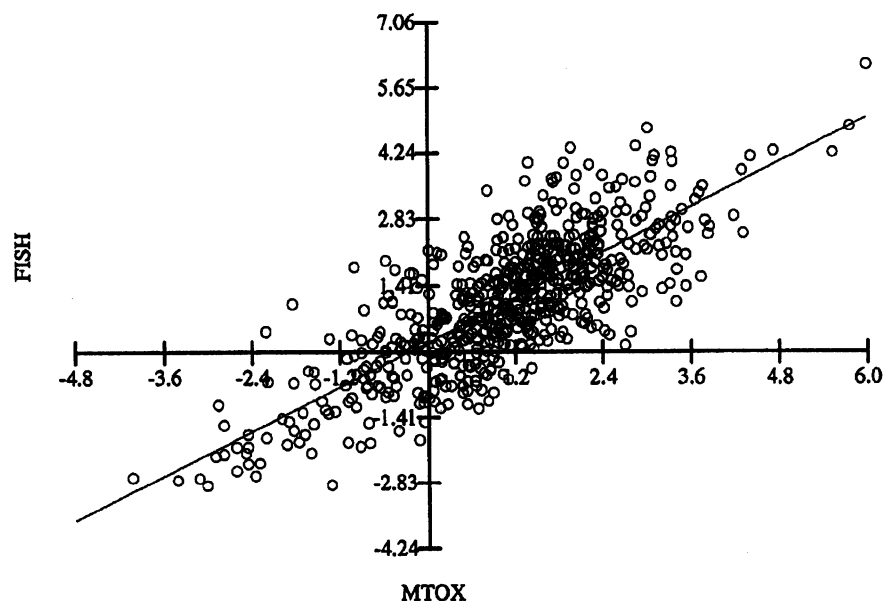

Figure 3. Plot of the acute toxicities of 635 individual organic chemicals for FISH-mix 96-hr LC_{50} values versus the luminescent bacteria 5-min to 30-min EC_{50} test (MTOX). Statistics are in Table 2; number of data trimmed, 12.

and a ciliate (*Tetrahymena pyriformis*), 48-and 60-hr LC_{50}. A comparison of the regression data in Table 4 with those for the fish (Table 2) shows generally similar slopes and standard deviations of the regressions. The somewhat higher standard deviations (1.15 to 1.65) for the crayfish and *Crangon* sp. can be explained by a preponderance of highly toxic substances (pesticides) in these data sets. The rather low slopes of 0.57 to 0.69 observed for the correlations with the *Tetrahymena* data are thought to arise from the high level of organic matter present in the culture medium for this organism, which leads to adsorption and complexation of the toxicants (*35*). The largest data set in this group, found for *D. magna*, covers 9 orders of magnitude in toxicity on a molar basis and leads to a highly significant correlation with a standard error of 0.85 pT units. Given the similarity of these equations with those for the fish, remarks concerning the applicability and accuracy of the predictive use of the regressions for fish apply to the algae and crustacean correlations as well. These data are entirely nonselected and obviously cover compounds of very different nature, from nonpolar narcotics to highly specific mode of action pesticides. Therefore they provide estimates for the average activity of chemicals, and if more accurate predictions are desired, more narrowly defined subsets are required.

## Correlations for Specific Groups of Substances

The highly promising results on interspecies toxicity correlations, as outlined above, raise the question as to their possible refinement by narrowing the applications to specific chemical classes or substances of the same mode of action or any other group that can be defined in a biochemical or chemical sense. Indeed, a plot of Microtox versus fathead minnow data for 18 halogen-free alcohol pairs shows an excellent relationship over

Table 4. Correlation of Microtox toxicity data with various other aquatic species toxicity data, all in pT units.

Species/time, hr	n	r	s	a	b	Range	Reference	Comments
Chlorella pyr./96	59	0.87	0.63	0.08	0.82	8	This paper	Trimmed (<2σ)
Daphnia magna/48	251	0.80	0.85	0.52	0.77	9	This paper	Trimmed (<2σ)
Tetrahymena pyr.	295	0.76	0.67	−0.41	0.65	8	This paper	Trimmed (<2σ)
Tetrahymena pyr./48	198	0.84	0.55	−0.46	0.69	8	This paper	Trimmed (<2σ)
Tetrahymena pyr./60	92	0.71	0.61	−0.44	0.57	4	This paper	Trimmed (<2σ)
Crayfish/96	31	0.68	1.65	0.88	1.05	5	This paper	Untrimmed
Crayfish/96	29	0.75	1.28	0.68	0.97	5	This paper	Trimmed (<2σ)
Artemia sal./24	63	0.90	0.65	0.16	0.73	9	This paper	Trimmed (<2σ)
Crangon sept./96	52	0.68	1.15	0.83	0.71	8	This paper	Trimmed (<2σ)

Table 5. Correlation of Microtox EC_{50} with fathead minnow 96-hr LC_{50} data for various groups of chemicals.

Chemical group	n	r	s	a	b	Range	Comments, conditions
Alcohols	22	0.97	0.36	−0.28	0.74	5.5	Halogen free
"Enol" phenols	43	0.76	0.68	0.13	0.83	4.5	No condition
"Ol" alcohols, phenols	88	0.90	0.66	−0.05	0.86	8	Trimmed (<2σ)
"Non" ketones	25	0.91	0.73	−0.13	1.00	5.5	No condition
"Ene" aromatics	66	0.85	0.70	0.00	0.91	7	Includes other compounds
"C6" compounds	83	0.85	0.77	−0.13	1.03	6	Untrimmed
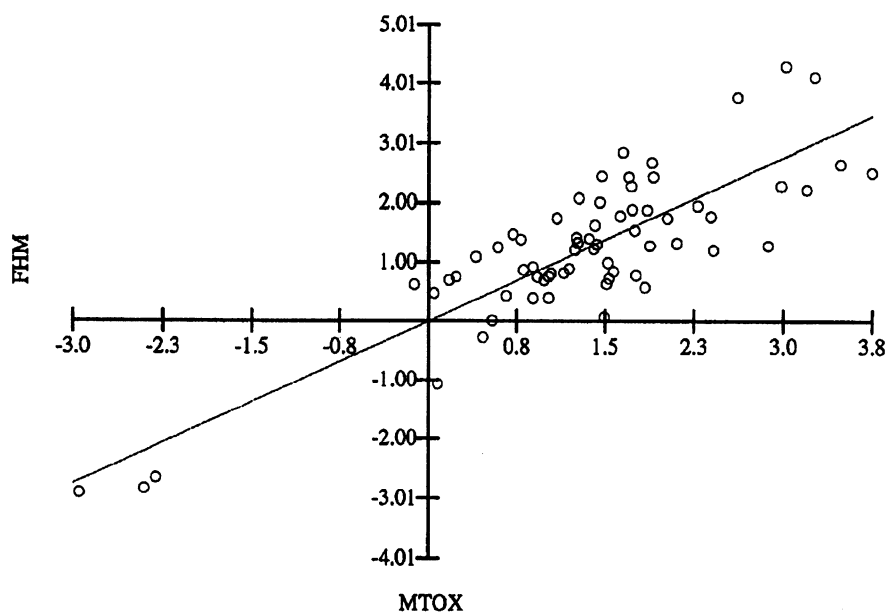
Figure 4. Plot of fathead minnow 96-hr LC_{50} values (FHM) for 66 compounds containing the name fragment "ene" versus *V. fischeri* EC_{50} values (MTOX). Statistics are in Table 5; number of data trimmed, 2.
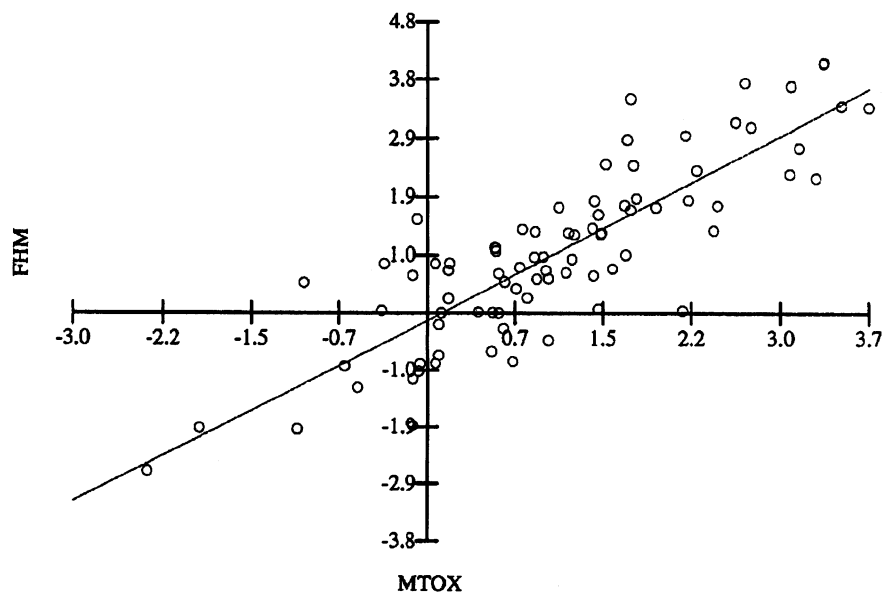
Figure 5. Plot of fathead minnow 96-hr LC_{50} values (FHM) for 83 compounds containing exactly six carbon atoms in the molecule versus *V. fischeri* EC_{50} values (MTOX). Statistics are in Table 5; number of data trimmed, nil.

approximately 6 orders of magnitude; the statistics are given in Table 5. Similar correlations can be developed for other groups for example compounds with the fragment "ol" in the name, i.e., mostly alcohols and phenols; compounds with the fragment "enol" in the name, primarily phenols; or compounds with the fragment "non" in the name, primarily ketones. Other examples of a simple selection of chemicals is the search for substances with the fragment "ene" in the name, mostly polynuclear aromatic hydrocarbons, the plot for which is shown in Figure 4. Another type of selection, based on the number of carbon atoms in the molecule, is shown in Figure 5 for compounds that have exactly six carbon atoms in the chemical formula (C6). The regression statistics for the above selections are given in Table 5 and it is apparent from both these graphs and regression models that highly significant correlations exist between the *V. fischeri* assay and the fathead minnow data for these groups of chemicals. Therefore, such selections can significantly improve the reliability of predicted values. Of course, similar analyses can be undertaken for each of the many species and end points for which the data are available to develop such models. Because there are also significant correlations between the *Vibrio* data and the bioassay results for other aquatic species, as shown in Tables 2 and 4, it is expected that these predictive models can be expanded to include several other species and toxicity end points.

Correlations with Toxicity Data for Terrestrial Species

There is a limited number of studies on the interspecies correlations of *Vibrio* bacteria toxicity data with those of terrestrial species, mainly rat and mouse, as well as some plants, earthworms, frogs, yeasts, and other bacteria (Table 1). In the latter, the data sets studied are too limited to draw any general conclusions. In contrast, for the mammalian species there is a large number (> 500 compounds) of data that have been investigated for the routes of

exposure, i.e., oral, intraperitoneal, and intravenous administration (7,10). The results show some general correlations, but the regression slopes are generally low and the standard errors of the estimates too high to be of much practical use, i.e., predictive capability, at this time. There is, however, a marked difference in the slopes of the oral, intraperitoneal, and intravenous routes of administration, with the oral route having the lowest slope and the intravenous route having the highest. This observation reflects the relatively higher degree of metabolization and detoxification or kinetically controlled limited uptake (especially for compounds with high lipophilicity and/or large molecular size) versus the intravenous route of exposure, which bypasses the intestine–blood barrier (10). For 171 chemicals, the correlation coefficient between mammalian (rat and mouse) intravenous median lethal dose (LD_{50}) and V. fischeri EC_{50} values was $r = 0.75$ with a standard error of 0.52 (10). Although this standard error is even smaller than for most of the correlations with the aquatic species shown above, the correlation's usefulness is limited because of the much smaller range of mammalian toxicity values in it. This problem is common to all attempts at correlating these mammalian LD_{50} data with physicochemical or other toxic response data; it is basically caused by the incongruent nature of these data rather than by the organism itself. Where compatible data are used, mammalian and bacteria data show quite close similarities in their response to the chemical challenge (6).

Practical Applications

There are numerous publications on compound-specific qualitative and quantitative comparisons of Vibrio bacteria test data with those for other organisms. Table 1 lists some of the major studies that deal with individual organic, inorganic, and organometallic compounds. In addition, there are many other publications on similar correlations of the toxic effects of

environmental extracts, such as leachates, sediment elutriates, surface and groundwater samples, complex effluents from industrial and municipal origins, treated and untreated waste materials, and others (36). Most authors of these reports recommend use of the bacterial test system as a component in a battery of tests to assess ecotoxicologic effects of complex effluents, contaminated sediments, and so forth. Where only a relative assessment is required, such as the measurement of spatial or temporal variation in a defined area or system, the use of the bacteria assay can be sufficient to delineate the gradient. In fact, some waste treatment authorities in Great Britain measure continuously the toxicity of incoming waste streams prior to treatment to prevent upset and maintain the biologic treatment system (37). In situations of sudden increase in toxicity (usually not identifiable from other measurements, such as biochemical oxygen demand), separation and special treatment are undertaken.

In terms of predictive applications to new chemicals or to those for which little else is known, extrapolation of the bacteria test to fish and other organisms can be done with varying degrees of confidence. The level of confidence for a new substance is a function of both the chemical complexity of the molecule in question and the availability of data for related compounds. For example, the effects of compounds with fewer and/or noninteracting functional groups can be predicted more readily and with a higher confidence than those for which electronic and steric conditions or unknown conformational changes may influence the properties and effects and potential specific interaction with receptor sites.

Conclusions

There are many highly significant interspecies relationships between Vibrio and numerous other aquatic species, which can be exploited to make confident predictions of one end point from another. This works particularly well for compounds

of relatively simple chemical structure with one reactive or functional group, such as the hydroxyl or keto moieties. As is the case with many other bioassays, chemicals of more complex structure, such as those with several functional groups interacting, with tautomeric and conformational changes, or with ionization occurring near physiologic pH values, are frequently associated with highly specific effects on a particular organism or biochemical function, and hence are more difficult to model. Although the available evidence suggests that there is a well known mean of effects for all compounds of similar polarity, molecular weight, size, functionality, etc., the potential deviation from this mean appears to increase with this complexity. As a result, less reliable predictions can be made for such substances, irrespective of whether basic physicochemical structure–activity relationships are used, such as correlations with the octanol/water partition coefficient or from interspecies correlations. There are also some noteworthy studies on Vibrio bacteria–mammalian toxicity relationships that indicate limited usefulness because of the nature of the mammalian data. However, the quality of these relationships increases markedly from oral LD_{50} to intraperitoneal LD_{50} to intravenous LD_{50} data. Therefore, future research efforts should be directed toward the latter type of mammalian data. Also, research into the development of multiple linear regression and neural net-type-based relationships is under way. In terms of speed, simplicity, and cost effectiveness, the Microtox test provides an excellent means of quickly measuring the acute toxic effect of either individual substances or complex mixtures on the Vibrio bacterium and, by way of proven inference, on a wide variety of higher organisms, particularly in the aquatic sphere. This test has become a standardized system for ecotoxicologic assessment in a variety of jurisdictions and is successfully being used to assess and improve practical treatment of effluents, soils, sediments, leachates, and other contamination problems.

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